

IN THE CLAIMS:

Please substitute the following amended claim number 27 for the pending claim having the same claim number.

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1. (Withdrawn)
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15. (Previously Amended) A method of selectively performing homologous recombination with a particular nucleotide sequence of a Bacterial or Bacteriophage-Derived Artificial Chromosome (BBPAC) that is contained in a recombination deficient host cell comprising introducing a conditional replication shuttle vector into a recombination deficient host cell and therein enabling homologous recombination in the host cell via the transient expression of a recombination protein in the host cell;

wherein the host cell comprises a Bacterial or Bacteriophage-Derived Artificial Chromosome (BBPAC) which contains the particular nucleotide sequence; wherein the conditional replication shuttle vector encodes a recombination protein that is transiently expressed by the host cell; wherein the conditional replication shuttle vector contains homologous nucleic acid sequences capable of selectively integrating into the particular nucleotide sequence when the recombination protein is expressed; and wherein the expressed recombination protein effectuates recombination of the shuttle vector and the Bacterial or Bacteriophage-Derived Artificial Chromosome (BBPAC).

16. (Previously Amended) A method of selectively modifying a particular nucleotide sequence of a Bacterial or Bacteriophage-Derived Artificial Chromosome (BBPAC) that is contained in a recombination deficient host cell comprising:

(a) introducing a conditional replication shuttle vector into a recombination deficient host cell; wherein the host cell comprises a Bacterial or Bacteriophage-Derived Artificial Chromosome (BBPAC) that comprises a gene of interest which contains the particular nucleotide sequence; wherein the conditional replication shuttle vector encodes a recombination protein that is expressed by the host cell and permits homologous recombination to occur in the host cell; wherein the conditional replication shuttle vector contains homologous nucleic acid sequences capable of selectively integrating into the particular nucleotide sequence when the recombination protein is expressed forming a co-integrate; wherein the nucleic acid sequences that selectively integrate into the particular nucleotide sequence and the nucleic acid encoding the recombination protein are positioned on the conditional replication shuttle vector such that upon resolution of the co-integrate, the nucleic acid encoding the recombination protein remains with the conditional replication shuttle vector; and

wherein the expressed recombination protein effectuates recombination of the shuttle vector and the Bacterial or Bacteriophage-Derived Artificial Chromosome (BBPAC); and

(b) growing the host cell under conditions in which the conditional replication shuttle vector cannot replicate, therein diluting out the conditional replication shuttle vector encoding the recombination protein, and thereby preventing further recombination events in the recombination deficient cells.

C, 17. (Original) The method of Claim 16 wherein the conditional replication shuttle vector further comprises a nucleic acid that encodes a marker protein or peptide and wherein the nucleic acid that selectively integrates into the particular nucleotide sequence and the nucleic acid encoding the marker protein or peptide are positioned on the conditional replication shuttle vector such that upon resolution of the co-integrate, the nucleic acid encoding the marker protein or peptide is inserted into or adjacent to the particular nucleotide sequence.

18. (Previously Amended) The method of Claim 16 wherein the conditional replication shuttle vector cannot replicate in the host cell because the conditional replication shuttle vector requires a particular protein for replication and neither the host cell nor the Bacterial or Bacteriophage-Derived Artificial Chromosome (BBPAC) encode the particular protein.

19. (Cancelled)

20. (Previously Amended) The method of Claim 16 wherein the BBPAC is a BAC or a PAC.

21. (Original) The method of Claim 20 wherein the conditional replication shuttle vector cannot replicate in the host cell because the conditional replication shuttle vector comprises a R6K $\gamma$  origin of replication and neither the host cell nor the BAC encode *pir*.

22. (Original) The method of Claim 21 wherein the conditional replication shuttle vector further comprises a first frt site that is positioned on one side of the nucleic acid that selectively integrates into the particular nucleotide sequence, and a second frt site that is positioned on the other side of the nucleic acid that selectively integrates into the particular nucleotide sequence and wherein the resolution of the co-integrate is performed by adding flip recombinase to the host cell.

23. (Original) The method of Claim 21 wherein the conditional replication shuttle vector further comprises a nucleic acid encoding a marker protein or peptide that is positioned in between the two frt sites and is also adjacent to the nucleic acid that selectively integrates into the particular nucleotide sequence such that after the resolution, the marker protein or peptide is contained by the BAC.

24. (Original) The method of Claim 22 wherein flip recombinase is added to the host cell by introducing a plasmid that encodes flip recombinase to the host cell.

25. (Original) The method of Claim 24 wherein the plasmid contains a conditional origin of replication.

26. (Original) The method of Claim 21 wherein the conditional replication shuttle vector further comprises two homologous nucleotide sequences that are homologous to each other but are not homologous to the BAC; wherein the two homologous nucleotide sequences are positioned on the conditional replication shuttle vector to be on opposite sides of the nucleic acid that selectively integrates into the particular nucleotide sequence; and wherein the resolution of the co-integrate is performed by a recombination event between the two homologous nucleotide sequences.

27. (Currently amended) The method of Claim 26 wherein the two homologous nucleotide sequences ~~are~~ encode a marker, IRESEGFP, which contains the ribosome entry site (IRES) and expresses enhanced green fluorescent protein (EGFP).

28. (Original) The method of Claim 21 wherein the recombination deficient host cell cannot independently support homologous recombination because the host cell is RecA<sup>-</sup>.

29. (Original) The method of Claim 21 further comprising adding a counterselection agent after the resolution of the co-integrate to remove host cells that comprise the conditional replication shuttle vector; wherein the conditional replication shuttle vector further comprises a counterselection gene that is positioned on the conditional replication shuttle vector such that upon resolution of the co-integrate the counterselection gene remains with the conditional replication shuttle vector.

30. (Original) The method of Claim 29 wherein the counterselection agent is sucrose and the counterselection gene is SacB.

31. (Original) The method of Claim 30 wherein the recombination deficient host cell cannot independently support homologous recombination because the host cell is RecA<sup>-</sup>.

32. (Original) The method of Claim 31 wherein the recombination protein is selected from the group consisting of recA, the rec E and rec T protein pair, the Lambda *beta* protein, and the *Arabidopsis thaliana* DRT100 gene product.

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48 (Previously Added). The method of claim 16, wherein said BBPAC is modified after resolution to result in nucleic acids insertions, and/or nucleic acids deletions, and/or point mutations on the BBPAC.

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